

ORIGINAL ARTICLE

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Comparison of the performance of serological kits for *Helicobacter pylori* infection with European and Asian study populations

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ABSTRACT

Most commercial kits for the detection of *Helicobacter pylori* were developed and validated with Western populations, and some have been found to perform less well with Asian populations. This study compared the performances of three serological kits with Swedish and Vietnamese peptic ulcer patients and asymptomatic individuals. The Pyloriset EIA-GIII and HM-CAP ELISA kits indicated that Asian populations had lower antibody titres to *H. pylori* than European populations. Despite the difference, the Pyloriset EIA-GIII kit performed well with Vietnamese peptic ulcer patients and population controls. The HM-CAP ELISA kit had a significantly lower performance with Asian populations that could not be improved by adjustments to the cut-off level. The Helicoblot 2.1 immunoblot kit performed equally well with Vietnamese and Swedish populations, although the response rate to the 35-kDa band was significantly lower with Vietnamese individuals.

Keywords Commercial kits, diagnosis, *Helicobacter pylori*, performance, target populations

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INTRODUCTION

Helicobacter pylori infects half the population of the world, causing chronic gastritis and peptic ulcer disease (PUD), and has also been associated with gastric cancer [1,2]. The infection can be confirmed by endoscopy, followed by culture of *H. pylori* from biopsies. Non-invasive tests to establish *H. pylori* infection, such as the urea breath test, serological assays and detection of antigen in faeces, are also used widely [3]. These tests have advantages, especially for studies in children and for epidemiological investigations. ELISAs for detection of *H. pylori* are based on antigens that are either whole-cell sonicates or one or more purified components of the bacter-

ium. Both types of assay exist in the form of in-house or commercially available formats.

It has been shown previously that the performance of in-house ELISAs can be improved for serodiagnosis of *H. pylori* infection in Asian populations by using local strains as antigens [4,5]. In addition, asymptomatic *H. pylori*-infected population controls have been shown to have lower IgG levels than PUD patients, necessitating a lower cut-off level for use in seroepidemiological studies [4,6]. Although an in-house ELISA is less expensive than a commercial kit, it presumes the availability of equipment and the knowledge necessary for large-scale culture of *H. pylori* for antigen preparation and the removal of cross-reactive antibodies [4]. Therefore, commercial serological kits are often the only option for serological diagnosis and seroepidemiological studies in developing countries. However, serological kits for the diagnosis of *H. pylori* infection were established and evaluated with Western

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populations. As a consequence, many kits have been found to perform less well with Asian populations [7–10].

The aim of the present study was to compare the performance of two commercial ELISA kits and an immunoblot kit with an in-house ELISA with European (Swedish) and Asian (Vietnamese) populations. The study also investigated whether the assays could be improved by adjusting the cut-off levels, thereby identifying the optimal commercial test for use with Asian populations.

MATERIALS AND METHODS

Study populations

The study populations comprised adults aged ≥ 18 years. Ninety-one Swedish gastric ulcer (GU) patients and 270 Vietnamese PUD patients, all of whom were positive for *H. pylori* by culture, had been identified previously in two separate treatment trials [11,12]. The population controls were 141 asymptomatic Swedish individuals, positive for *H. pylori* by the ^{14}C urea breath test, which has a sensitivity of 93% and a specificity of 100% [13], and 429 Vietnamese individuals who were diagnosed as *H. pylori*-positive or -negative by the immunoblot kit, which was considered to be the reference standard following evaluation in the other three groups, as the urea breath test was not available in Vietnam [4].

Diagnostic kits and in-house ELISA

The commercial kits used were Pyloriset EIA-GIII (Orion Diagnostica, Espoo, Finland), HM-CAP (Enteric Products, Westbury, NY, USA) and Helicoblot 2.1 (Genelabs, Singapore). The Pyloriset EIA-GIII contains a partially purified protein preparation of *H. pylori* collection strain NCTC 11637. A single cut-off level of 20 ELISA U/mL is recommended by the manufacturer for the Pyloriset EIA-GIII. In order to compare the results of this assay with those of the HM-CAP and the in-house ELISA, both of which normally allow $\pm 10\%$ around the cut-off, single cut-off values were also used for these assays. The HM-CAP kit, containing high molecular mass cell-associated antigens, recommends that ELISA values < 1.8 should be regarded as negative results, those of 1.8–2.2 as intermediate, and values > 2.2 as positives. The single cut-off value was set at 2.0. For the in-house ELISA, local strains (i.e., from Swedish and Vietnamese populations, respectively) were used in conjunction with the common cut-off level established previously at OD₄₀₅ 0.22 [4]. The Helicoblot 2.1 immunoblot kit contains *H. pylori* collection strain ATCC 49503, supplemented with a recombinant antigen designated 'current infection marker' (CIM) [14]. The immunoblot kit was evaluated according to the instructions of the manufacturer, with single bands at 35 kDa, 37 kDa and 89 kDa (VacA), and with combinations of these bands as the CIM, 19 kDa, 30 kDa (Urease A) and 116 kDa (CagA) all being diagnostic for *H. pylori* infection.

Statistical analysis

Sensitivity and specificity were calculated with exact binomial 95% CIs (STATA v. 8.0; Stata Corp., College Station, TX, USA). Serological results were presented by reverse cumulative distribution plot, as recommended previously for serological data [15]. These curves allow results to be reviewed over the entire range of absorbance values, with the shape of the curves indicating the likelihood of intermediate results around a given cut-off level. Receiver operating characteristic (ROC) curve plots of test sensitivity and test specificity at different cut-off levels were compared to evaluate and present test performances [16].

Ethical approval

The study design and materials were approved by the Ethics Committee of Karolinska University Hospital in Sweden (No. 98-260, 154 : 1/99, 02-031 and 96/063) and the Ethics Committee of the National Institute of Hygiene and Epidemiology in Vietnam.

RESULTS

All four assays showed high sensitivity levels of 96.7–100% with the Swedish populations and the Vietnamese PUD patients, with the exception of the HM-CAP kit, which was significantly ($p < 0.001$) less sensitive than the other assays (Table 1). With the Vietnamese population controls, and the immunoblot as reference, the in-house ELISA and the Pyloriset EIA-GIII kit performed well, while the HM-CAP kit had a lower sensitivity and specificity (Table 2). A cause for the discrepant results is illustrated by Fig. 1(A–C), which shows lower antibody titres in the Vietnamese populations compared with the Swedish populations. The lower IgG titres were particularly noticeable in the Vietnamese population controls.

In order to improve the performance of the assays, ROC curves were constructed for the Pyloriset EIA-GIII and HM-CAP kits (Fig. 2), as constructed previously for the in-house ELISA [4]. The data indicated that the performance of the Pyloriset EIA-GIII kit could be improved further with the two Vietnamese populations by slightly lowering the cut-off level to 19, while the performance of the HM-CAP kit at the indicated optimal cut-off level of 1.8 remained poor.

The performance of the immunoblot (Helicoblot 2.1 kit) was also investigated with the two populations (Fig. 1D). A major difference was noted only for the antibody response to the 35-kDa antigen, where a response was significantly ($p < 0.001$) less frequent with the Vietnamese

Population	Sensitivity of assay (%)			
	In-house ELISA 95% CI (n/n total)	Pyloriset EIA-GIII 95% CI (n/n total)	HM-CAP 95% CI (n/n total)	Helicoblot 2.1 95% CI (n/n total)
Culture-positive Swedish GU patients	97.8 92.3–99.7 (89/91)	96.7 90.7–99.3 (88/91)	97.8 92.3–99.7 (89/91)	98.9 94.0–100 (90/91)
UBT-positive Swedish asymptomatic patients	99.3 96.1–100 (140/141)	99.3 96.1–100 (140/141)	100 97.4–100 (141/141)	100 97.4–100 (141/141)
Culture-positive Vietnamese PUD patients	99.6 98.0–100 (269/270)	98.5 96.3–99.6 (266/270)	91.5 87.5–94.5 (247/270)	99.6 98.0–100 (269/270)

GU, gastric ulcer; UBT, urea breath test; PUD, peptic ulcer disease.

Table 1. Sensitivity of in-house ELISA, Pyloriset EIA-GIII, HM-CAP and Helicoblot 2.1 kits for serodiagnosis of *Helicobacter pylori* infection

Table 2. Sensitivity and specificity of the ELISA assays with Vietnamese population controls, with the Helicoblot 2.1 kit as the reference standard method

Sensitivity	Western blot (+) (n = 375)	
	% positives in assays	95% CI
ELISA (in-house)	93.9	90.9–96.1
Pyloriset EIA-GIII	84.8	80.8–88.3
HM-CAP	77.6	73.0–81.7
Specificity	Western blot (–) (n = 54)	
	% negatives in assays	95% CI
ELISA (in-house)	90.7	79.7–96.9
Pyloriset EIA-GIII	94.4	84.6–98.8
HM-CAP	88.9	77.4–95.8

populations in comparison with the Swedish populations. As this band alone is diagnostic for *H. pylori* infection, a lower rate of response to this antigen among Asians could decrease the sensitivity of the immunoblot for Asians compared with Europeans. However, the performance of the immunoblot was excellent with both populations, as an isolated response to this band was not noted with any of the study populations.

DISCUSSION

The present study demonstrated a variable performance of the serological kits used for serodiagnosis of *H. pylori* infection among products, among populations (i.e., European vs. Asian), within these populations, and among subjects with asymptomatic infection and those with PUD. In the Vietnamese populations, asymptomatic individuals had lower antibody titres than PUD patients by all the ELISA-based assays included in

the study. The same observation has been reported previously for the in-house ELISA [4]. This difference was not seen with the asymptomatic Swedish patients and the Swedish GU patients. The most plausible explanation would seem to be that GU patients have less inflammatory response, and possibly more atrophy, than PUD patients, of whom the majority had duodenal ulcer (DU), some had DU and GU, and only a few had GU.

In order to test this hypothesis, the in-house ELISA results obtained with 158 *H. pylori* culture-positive Swedish DU patients from another trial [17] were compared with the two Swedish study populations in the present study (data not shown). The DU patients had significantly higher antibody concentrations than the other two populations, with the magnitude of the difference being even more pronounced than with the Vietnamese study populations. The important difference in the height of the antibody response between DU patients and asymptomatic individuals (and GU patients) has relevance for the choice of cut-off level. If *H. pylori* culture-positive DU patients are used as the reference, the cut-off level might be set too high for screening purposes and for seroepidemiological studies. The difference might also explain, in part, some of the divergent results obtained in evaluations of commercial kits with Asian populations, in which the kits perform well with DU patients, but less so with other *H. pylori*-infected groups.

The performance of the HM-CAP kit was equally good with symptomatic and asymptomatic Europeans, but was inferior with the Asian populations when compared with the other methods investigated (i.e., in-house ELISA with strains from the study population, the Pyloriset

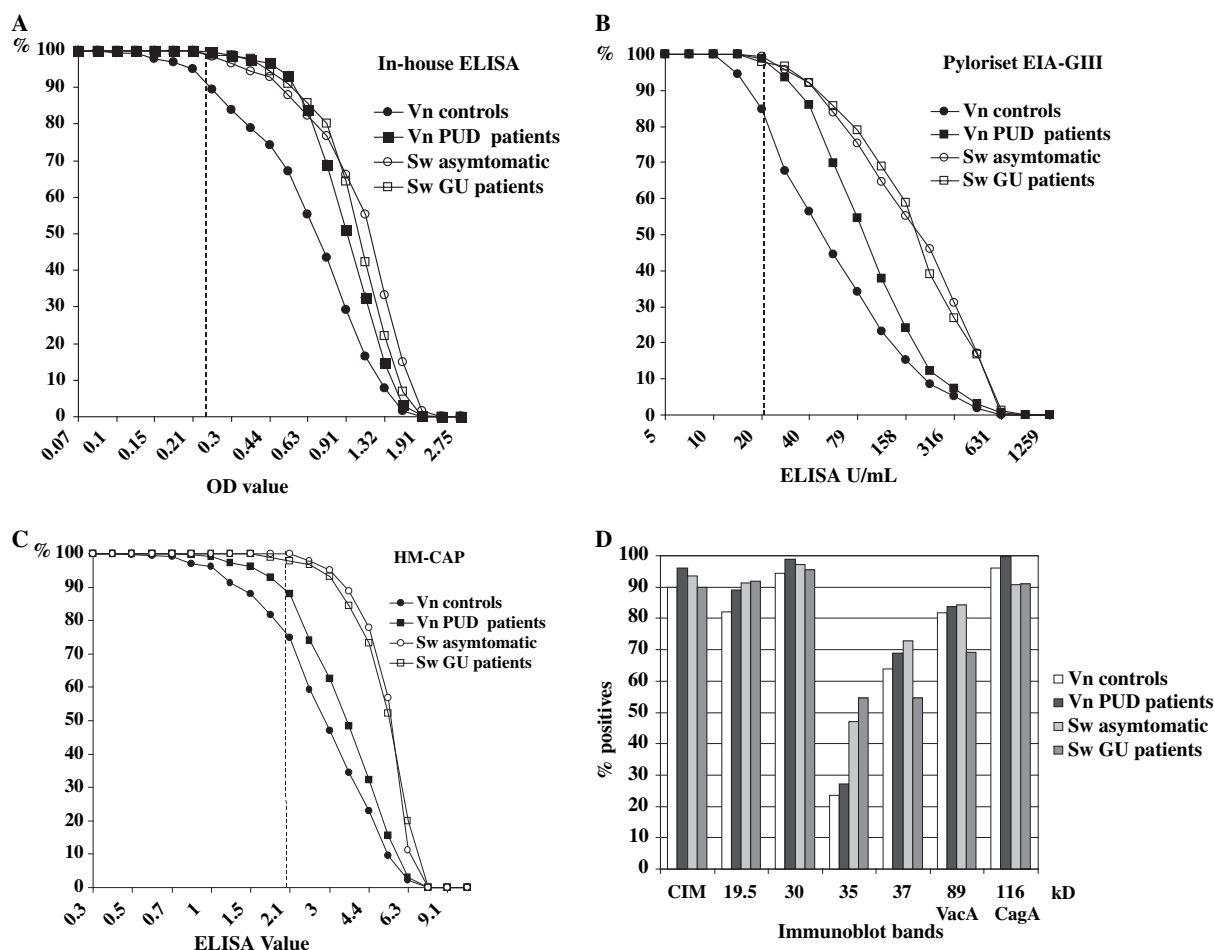


Fig. 1. A–C, Reverse cumulative graphs of antibody concentrations in the ELISA assays. The dotted lines represent cut-off levels in the respective assays. D, Proportion of responders to the diagnostic bands in the Helicoblot 2.1 kit (Vn, Vietnamese; Sw, Swedish; PUD, peptic ulcer disease; GU, gastric ulcer).

EIA-GIII and the Helicoblot 2.1 immunoblot kits). The performance of the HM-CAP kits with Asian populations could not be improved significantly by adjustment of the cut-off level. The results thus confirm the observations of Marchildon *et al.* [10] of major differences among Asian and European strains with respect to the high molecular size cell-associated proteins used as antigens in this kit.

The Pyloriset EIA-GIII kit performed well with all four study groups (i.e., Swedish asymptomatic *H. pylori*-infected individuals, Swedish GU patients, Vietnamese immunoblot-positive asymptomatic population controls and Vietnamese PUD patients). The performance of an earlier version of this kit was reported to be less than optimal with a Chinese population [7]. However, the current version of the kit showed the same high degree of sensitivity with Thai dyspeptic patients [18] as

found in the present study. In the Thai study, all the kits tested had a low specificity, but these determinations suffered from the difficulties entailed when trying to identify true-negatives, especially in an Asian setting. When using the immunoblot as a reference standard in the present study, the specificity of the Pyloriset EIA-GIII kit was high with the Vietnamese population controls.

The Helicoblot 2.1 immunoblot kit has been evaluated previously for sensitivity and specificity with Japanese and American populations with respect to the putative virulence factors CagA (89 kDa) and VacA (119 kDa), and has yielded good results [14]. The present evaluation of all the bands showed that the kit performed equally well with respect to sensitivity with European and Asian populations. The only significant difference was the lower response rate to the 35-kDa protein

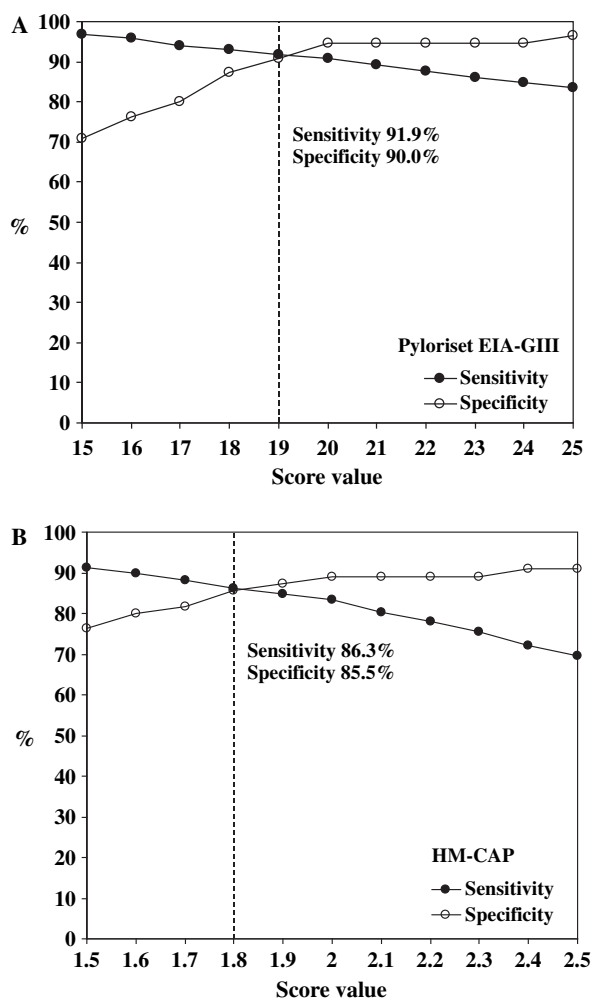


Fig. 2. Receiver operating characteristic (ROC) curves for (A) Pyloriset EIA-GIII and (B) HM-CAP ELISA values with the Vietnamese study populations, as used for the establishment of an optimal cut-off level.

with the Vietnamese study populations, but this did not impact negatively on the sensitivity of the kit.

In conclusion, this study demonstrated that Vietnamese study populations have lower antibody responses to the *H. pylori* antigens contained in commercial kits than do Swedish populations. Despite this difference, the commercial Pyloriset EIA-GIII kit performed well with Asian populations, and the performance of the kit could be improved further by a slight lowering of the cut-off level. In contrast, the HM-CAP kit showed a poor performance that could not be improved significantly by adjusting the cut-off level. The Helicoblot 2.1 immunoblot kit performed equally well with all the populations tested, despite a

difference in the response rate to one band between Swedish and Vietnamese individuals.

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